



Risk of Therapeutic Failure Due to Ineffectiveness of Medication

Pharmacology Discipline Standing Review

October 10, 2011

SK3/Wotring/3-6332

Risk Statement



Given that terrestrial medical practices must be used as the basis for drug choice and use on missions, there is a possibility that medications used will be ineffective or inappropriate for the actual circumstances encountered on missions.

Risk Context

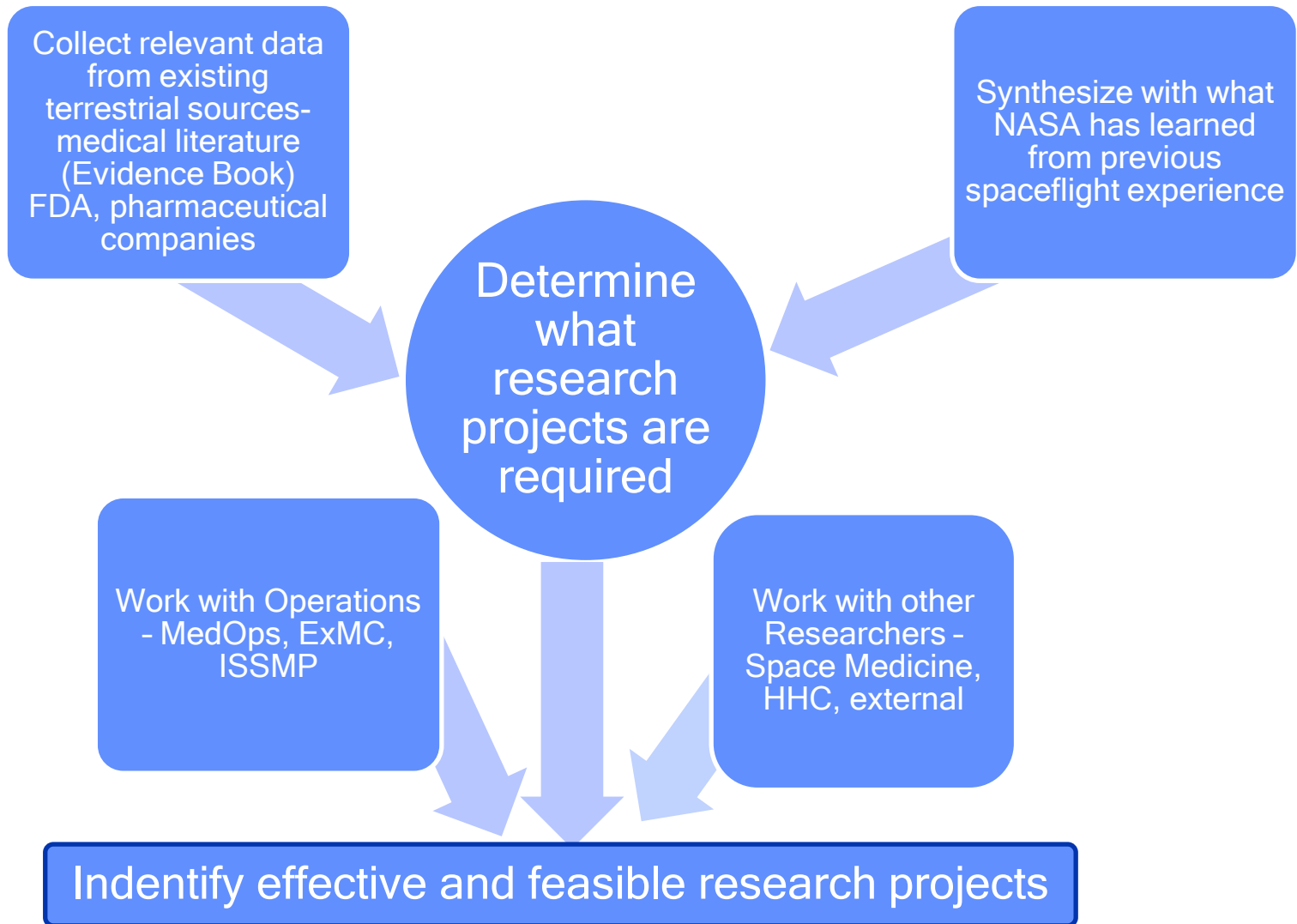


Because the human body undergoes a variety of physiological changes during spaceflight, there is a risk that terrestrial medications may not perform as expected when used during spaceflight.

Alterations in physiology due to spaceflight could result in unexpected drug action on the body (pharmacodynamics) or in unusual drug absorption, distribution, metabolism or excretion (pharmacokinetics).

The spaceflight environment may also have direct effects on stored drugs themselves, leading to premature inactivation or degradation of stored drugs.

Research Plan - the approach



Pharmacology SRP Suggestions:



“Prioritization of Pharmacology-Risk Activities:

Based on available evidence, pharmacology research activities in the near future should be prioritized towards the following endeavors:

1. Evaluation of Pharmacokinetics/Pharmacodynamics (PK/PD) of antibacterials/antivirals;
2. Assessment of long-term spaceflight on drug distribution including *in-vivo* drug metabolism and renal drug clearance;
3. Risk assessment of promethazine-induced QT_c prolongation;
4. Testing of shelf-life/potentially protective packaging of prototypical medications (e.g., L-thyroxine) in ground-based studies.”

Revisions from IRP Rev B to Rev C



Revisions from IRP Rev B to Rev C were intentionally kept to a minimum, because of the comprehensive IRP-wide Gap re-write scheduled for later in 2011. One redundancy in the set of Pharmacology Gaps was corrected. Two similar Gaps across Disciplines were merged.

- Removed Gap PH11; Redundant with Gap PH 2
 - Deleted: PH11: What potential polypharmacy problems exist in current spaceflight medical practice?
 - Kept: PH02: What drug interactions may adversely affect clinical care?
- Merged Gaps- Integrated Gap SM25 into PH10
 - Deleted: SM25: What are the multisystem effects of promethazine? (a Sensorimotor Gap)
 - Kept: PH10: What are the performance effects of in-flight drugs on exercise, orthostatic tolerance, motor control, cognitive function, or other performance-determining aspects of physiology? Can novel multidisciplinary therapeutic approaches be used to enhance efficacy and reduce side effects?

Gaps



PH01: Inadequate tracking of medication use, indication, efficacy, and side effects.

PH02: What drug interactions may adversely affect clinical care? **Clinical Pharmacy**

PH04: What diagnostic, therapeutic, and laboratory technologies are necessary to predict and manage medication side effects, interactions, and toxicity during spaceflight? **Hardware**

PH06: Can a standard procedure for prospective analyses of drugs to be considered for flight and periodic analyses of drugs that are used for flight be developed? **Space Medicine**

PH07: What are the effects of spaceflight on pharmacokinetics and pharmacodynamics?

PH09: What is the stability of drugs during long duration spaceflight?

PH10: What are the effects of in-flight drugs on exercise, orthostatic tolerance, motor control, cognitive function, or other performance-determining aspects of physiology? Can novel multidisciplinary therapeutic approaches be used to enhance efficacy and reduce side effects?

PH13: Which sleep aid is best in flight in terms of efficacy? In terms of limited side effects?
BHP

PH15: Are the antimicrobials carried on board effective against microbes that exhibit spaceflight-related changes?

Gaps



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PH15: Are the antimicrobials carried on board effective against microbes that exhibit spaceflight-related changes?

NASA Research Announcement 8/2011



Proposals are solicited by NASA in the areas of:

Visual Acuity and Ocular Structure and Function; Fluid Distribution; Team Social, Technical, and Task Roles; and Effects of Constrained, Asynchronous Communication on Operational Tasks.

In addition, NASA is requesting short-term investigations (< 1yr and < \$100K) that provide innovative approaches to any of the defined risks contained in the Integrated Research Plan (IRP) of the Human Research Program.

The NSBRI portion of this NRA solicits for:

Microgravity-Induced Visual Alterations and Intracranial Pressure, Cardiovascular Alterations, Human Factors and Performance Musculoskeletal Alterations, Neurobehavioral and Psychosocial Factors, Sensorimotor Adaptation, Smart Medical Systems and Technology

Research Plan Top Priorities

(in order of importance and urgency)



- 1. Improve information about inflight medication efficacy and side effects**
- 2. Improve knowledge of drug stability during spaceflight**
- 3. Prepare for virulence changes in space**
- 4. Develop a better understand of spaceflight effects on metabolism and radiation-induced oxidative damage**
- 5. Gather basic PK information to better inform dosing**
- 6. Investigate therapies for SMS**

Research Plan Top Priorities

(in order of importance and urgency)



1. **Improve information about inflight medication efficacy and side effects** Collect data on **current** inflight medication use efficacy and side effects. Collect existing data from NASA data archives.

Directly addresses:

PH01 Inadequate tracking of medication use, indication, and side effects.

Medication Symptom Tracking Questionnaire - collect research information about inflight drug use going forward (proposal submitted 9/2011)

Limitations- requires voluntary crew participation, most flight surgeons feel collection of research information is unnecessary and burdensome on crew

Data Mining from LSDA/LSAH, ExMC, BHP, NSBRI, FDA

Limitations- data are sparse; there is no indication of medication efficacy; access to NASA data sets is very limited, intensive data analysis requires significant personnel time

Will also inform all other PH Gaps, particularly:

PH10 What are the effects of in-flight drugs on exercise, orthostatic tolerance, motor control, cognitive function or other performance-determining aspects of physiology?

Deliverables: Hard data about medication use frequency as well as identification and frequency of untoward effects; will inform Gaps in other Disciplines, Space Medicine, BHP, ExMC.

Research Plan Top Priorities

(in order of importance and urgency)



2. Improve knowledge of drug stability during spaceflight

1. Conducting in-house data mining to collect and analyze unpublished data from previous spaceflight analog and spaceflight experiments.
2. In process of negotiating data sharing arrangements with FDA and pharmaceutical companies to get as much information as possible from existing terrestrial sources.
3. Arranging a pilot study with the FDA stability labs, in which they will conduct a comprehensive battery of tests of some returned flight medications.

Directly addresses:

PH09 What is the stability of drugs during long duration spaceflight?

Also addresses specific SRP concerns.

Deliverables: Information will be used to determine future research needs for long duration mission planning.

Research Plan Top Priorities

(in order of importance and urgency)



- 3. Prepare for virulence changes in space:** Determine the efficacy of antibiotics and antifungals in ground analog.

Proposal submitted 9/2011, in conjunction with JSC Microbiology (Mark Ott SF/NASA)

3 microorganisms found on the ISS

(Pseudomonas, Staph aureus, Salmonella enterica serovar Typhimurium)

grown in a culture system that mimics microgravity

measuring mean inhibitory concentration of 2 medications used to combat infections by each organism



Research Plan Top Priorities

(in order of importance and urgency)



- 3. Prepare for virulence changes in space:** Determine the efficacy of antibiotics and antifungals in ground analog.

Limitations: Omnibus Proposal is limited to 1 yr and \$100K; microbial changes could be transient, requiring a faster protocol than typically used for MIC experiment; different microorganisms may not behave the same way in the model system.

Directly addresses:

PH15 Are the antimicrobials agents carried on board effective against microbes that exhibit spaceflight-related changes?

Also addresses specific SRP concerns.

Deliverables: Will deliver pilot information that will help determine the scope of any potential problem, develop methods and validate the rotating culture system for pharmaceutical testing, and provide a framework for more comprehensive studies in the future. Information regarding antimicrobial efficacy (including required dosages) would ultimately inform Space Medicine's treatment decisions.

Research Plan Top Priorities

(in order of importance and urgency)

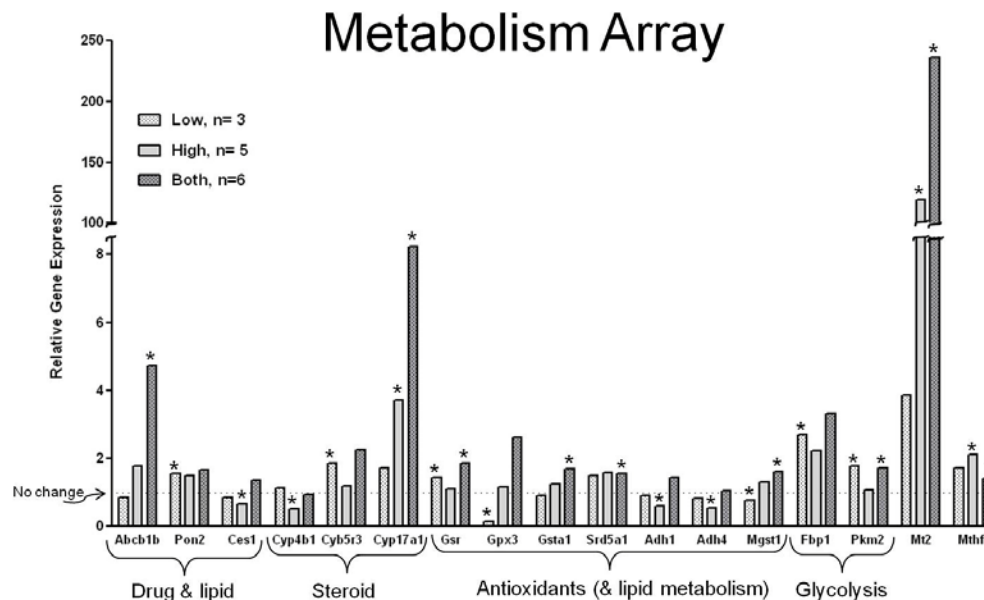


5. Gather basic PK information to better inform dosing:

Pilot Study - Liver function in mice after radiation exposure

A specimen-sharing add-on study

Groups of 6 mice were exposed to 50 mGy, or 6 Gy, or 50 mGy followed by 6 Gy. RNA was extracted from liver samples.



Research Plan Top Priorities

(in order of importance and urgency)



5. Gather basic PK information to better inform dosing:

Pilot Study - Liver function in mice after radiation exposure

Limitations: mice are not humans; single high dose exposures may cause different effects than chronic low dose exposure; because this was an add-on to someone else's study, no drug administration was possible; radiation alone may not adequately reproduce the spaceflight environment; changes at the RNA level may not reflect activity at the protein level.

Directly addresses:

PH7 What are the effects of spaceflight on pharmacokinetics and pharmacodynamics?

Also addresses specific SRP concerns.

Deliverables: Radiation exposures can alter expression of genes involved in metabolism of administered medications. The same radiation exposures also alter express of gene involved in redox homeostasis.

Research Plan Top Priorities

(in order of importance and urgency)



4. Develop a better understand of spaceflight effects on metabolism and radiation-induced oxidative damage

Ground based animal study in progress: Pilot Studies of Radiation Damage in Organ Tissues of Mice (previous 2 slides)

Flight animal study in progress - Effect of Spaceflight on Expression of Metabolic Enzyme Genes in Mice

permitted access to livers from mice who experienced a shuttle flight (through the Biological Specimen Sharing Program)

will perform gene expression studies similar to the Radiation Damage study just described, as well as protein analyses

Limitations: mice are not humans; exhaustive tissue sharing arrangements increased the length of time between animal sacrifice and liver dissection – tissue quality may be somewhat compromised; an add-on study so no drugs could be administered

Research Plan Top Priorities

(in order of importance and urgency)



4. Develop a better understand of spaceflight effects on metabolism and radiation-induced oxidative damage



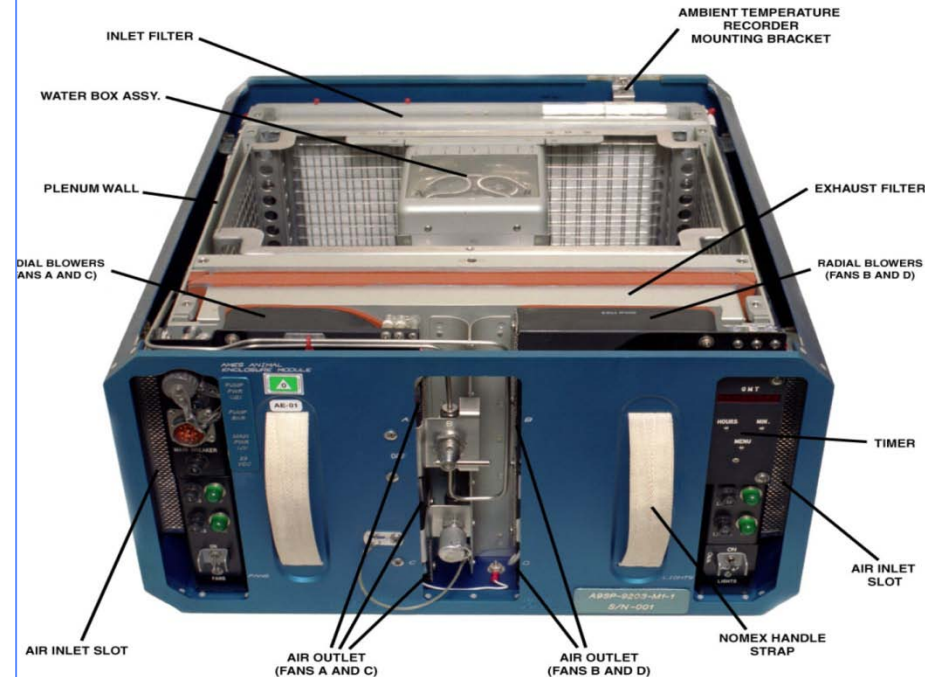
Study in progress - Effect of Spaceflight on Expression of Metabolic Enzyme Genes in Mice

Directly addresses:

PH7 What are the effects of spaceflight on pharmacokinetics and pharmacodynamics?

Also addresses specific SRP concerns.

Deliverables: Information regarding the effects of spaceflight on liver gene and protein expression, which will narrow the focus for future research.



Research Plan Top Priorities

(in order of importance and urgency)



4. Develop a better understand of spaceflight effects on metabolism and radiation-induced oxidative damage

Ground based radiation+oxidative stress proposal submitted - Effects Of Radiation And Dietary Iron On Expression Of Genes And Proteins Involved In Drug Metabolism – proposal submitted 9/2011

Another study from the Nutritional Biochemistry Discipline used rats with a 3 Gy radiation dose delivered in several session over 3 weeks. Some animals were given a high iron diet.

will perform gene and protein expression studies similar to the flight study just described

Limitations: rodents are not humans; because this was an add-on to someone else's study, no drug administration was possible

Directly addresses:

PH7 What are the effects of spaceflight on pharmacokinetics and pharmacodynamics?

Also addresses specific SRP concerns.

Deliverables: Information regarding the effects of spaceflight on liver gene and protein expression, which will narrow the focus for future research. Validation of rat model with lower, fractionated dose radiation exposure.

Research Plan Top Priorities

(in order of importance and urgency)



6. Investigate therapies for SMS:

Funded proposal –

Effect of Anti-Motion Sickness Medication Combinations on QT_c Interval

Limitations: Will take considerable time to have 50 subjects complete this study; this study does not include any efficacy measures.

Directly addresses:

PH10 What are the effects of in-flight drugs on exercise, orthostatic tolerance, motor control, cognitive function, or other performance-determining aspects of physiology? Can novel multidisciplinary therapeutic approaches be used to enhance efficacy and reduce side effects?

Also addresses specific SRP concerns regarding the potential for cardiac side effects with promethazine.

Deliverable: Measurements of cardiac activity collected during SMS treatments; an indicator of medication safety. Depending on result, could provide evidence for replacement of certain medications or for use of one medication combination over another.

Research Plan Top Priorities

(in order of importance and urgency)



6. Investigate therapies for SMS:

Funded project –

Effect of Anti-Motion Sickness Medication Combinations on QT_c Interval

Virginia Wotring, JSC SK/USRA, James P. Locke, JSC/SD and Todd T. Schlegel, JSC/SK

to compare the safety of two new combination medication treatments for motion sickness with 1) other medication treatments already in use and 2) an inactive (placebo) control.

will compare the effect on QT_c, R-R and other measures derived from high resolution 12-lead ECGs.

each of the 50 subjects will participate in 5 separate sessions, with each session involving the ingestion of a different combination of medicines (or placebo) plus the performance of an ECG and side effect questionnaire before medication, 90 minutes after medication administration, and 24 hours after.

- 1) scopolamine/modafinil/ondansetron
- 2) promethazine/modafinil/ondansetron
- 3) scopolamine/dextroamphetamine
- 4) promethazine/dextroamphetamine
- 5) placebo

Back Up Slides



Contributing Factors (Master Logic Diagram - MLD - new version)



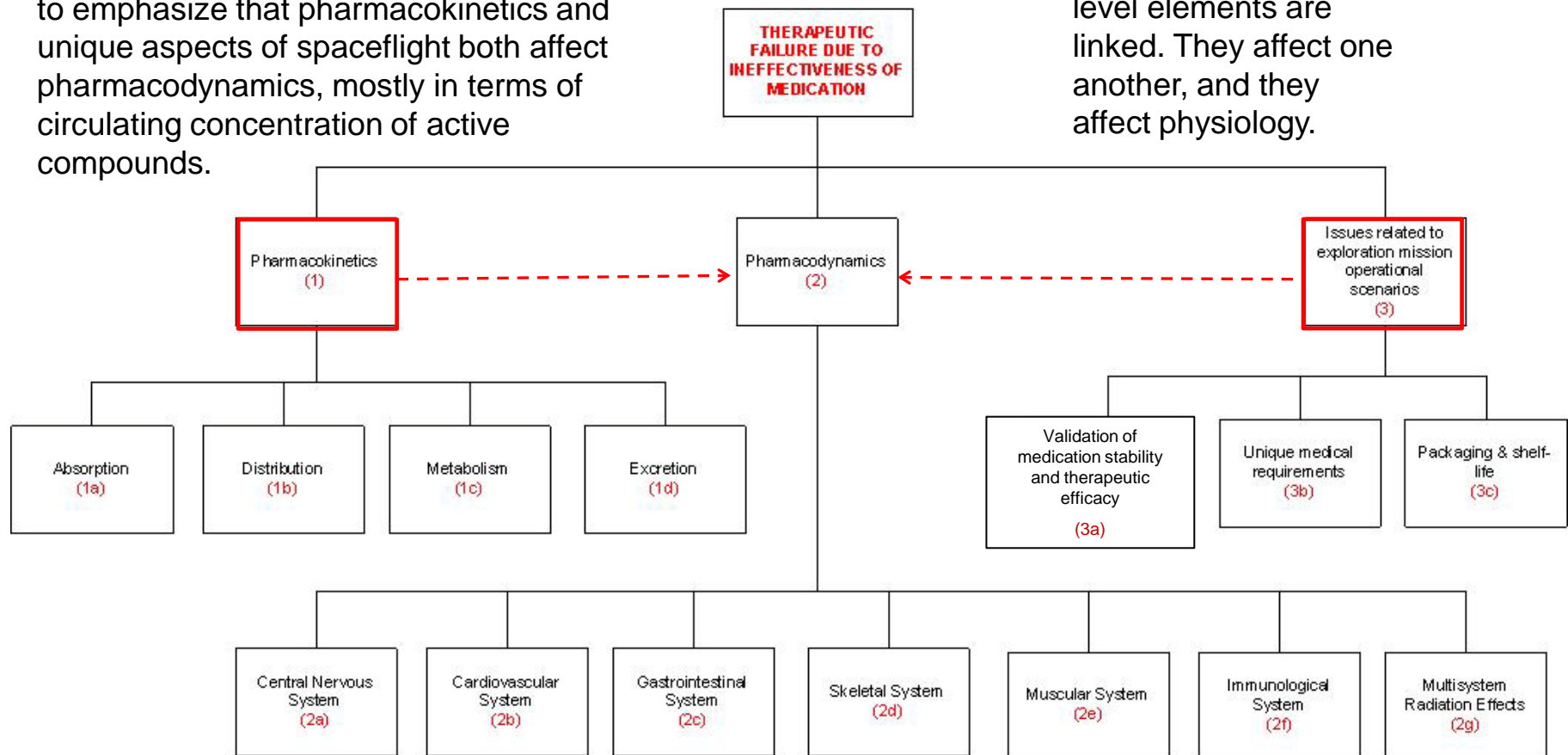
As of 9/10

Some confusion was expressed regarding the connection of the 3 main elements. We have revised the diagram to emphasize that pharmacokinetics and unique aspects of spaceflight both affect pharmacodynamics, mostly in terms of circulating concentration of active compounds.

MLD - Version 5
(Iteration 2)

Risk of Therapeutic Failure Due to
Ineffectiveness of Medication

All three of these top level elements are linked. They affect one another, and they affect physiology.



Drugs are metabolized by enzymes, which are proteins.

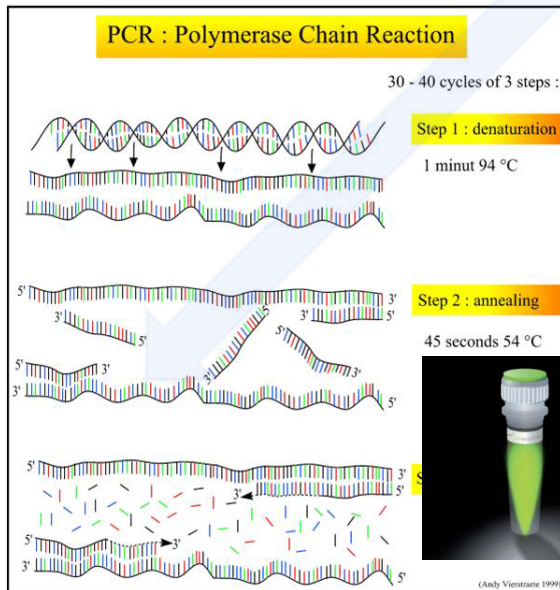


remember that DNA → RNA → protein

which means that experiments can probe RNA expression levels or protein expression/function

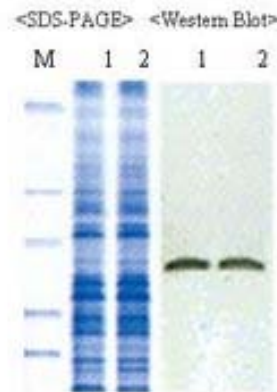
Gene expression

PCR— can examine 96 mRNAs simultaneously



Protein expression

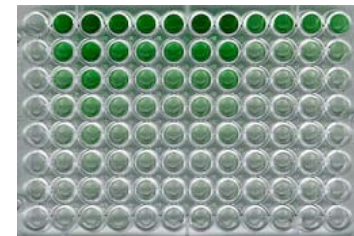
Western blot— requires unique antibody for every protein



SRF 2011

Protein function

Enzyme assay — requires unique assay for every enzyme



Livers were removed from the anesthetized animals immediately after sacrifice, and the livers flash-frozen in liquid nitrogen. Tissue will be homogenized, RNA extracted, and purified. Quality of RNA samples will be evaluated with the Agilent system in the HACD Core Lab. Complementary DNA will be prepared from the RNA samples, and used to run commercially available RT-PCR screening arrays for DNA Repair and Drug Metabolism (SuperArray, SABiosciences, Qiagen). Use of these large pre-optimized primer sets for this pilot experiment will allow efficient screening of over 150 gene products using the BioRad Cfx96 Real-time PCR System in the HACD Core Lab. The results from this phase of the experiment should allow us to not only determine if radiation exposure affects expression level of genes coding for these enzymes, but also to determine if particular groups of enzymes are affected more than others. For example, we might see enzymes that repair breaks in double-stranded DNA upregulated by radiation exposure, while those that repair DNA mismatches or transport drugs across membranes are relatively unaffected, allowing us to focus future research effects to the most important targets.

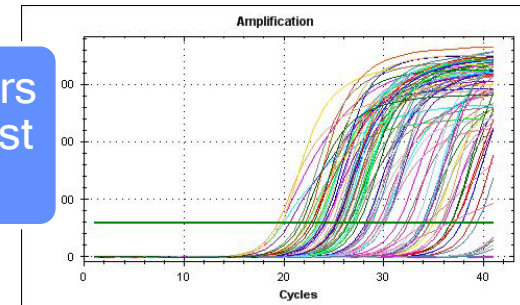
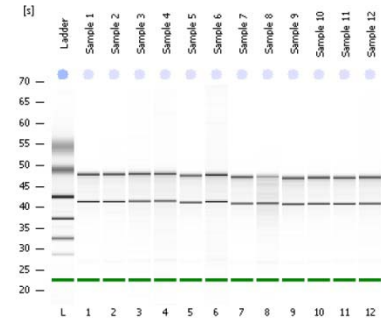
Extract RNA from tissue

Verify quantity and quality of RNA

Make cDNA to use as template for PCR

Perform PCR using primers coding for genes of interest and reference genes

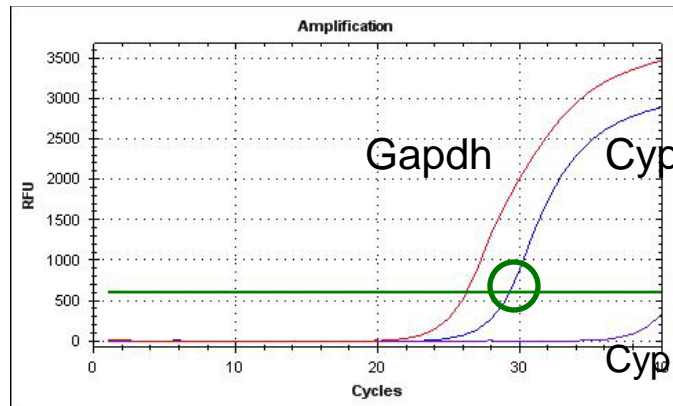
Calculate gene expression relative to reference genes



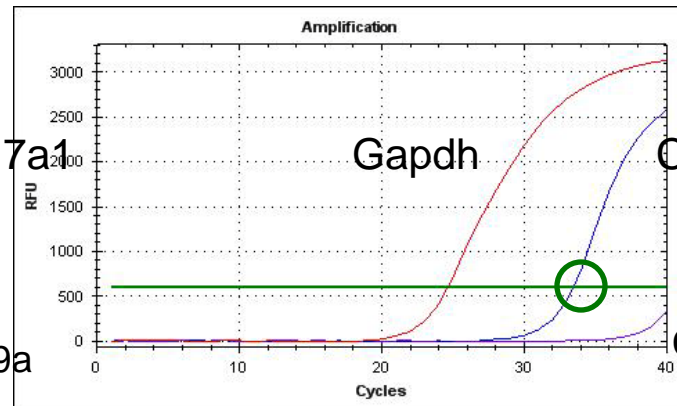
4 hour experiment - lab analysis complete & statistical analysis underway

24 hour experiment - RNA extractions complete

Ct shifts allow quantitation of expression levels relative to reference genes



Mouse that was exposed to both radiation treatments



Control animal

This Ct shift means a 7-fold increase in expression of this gene.

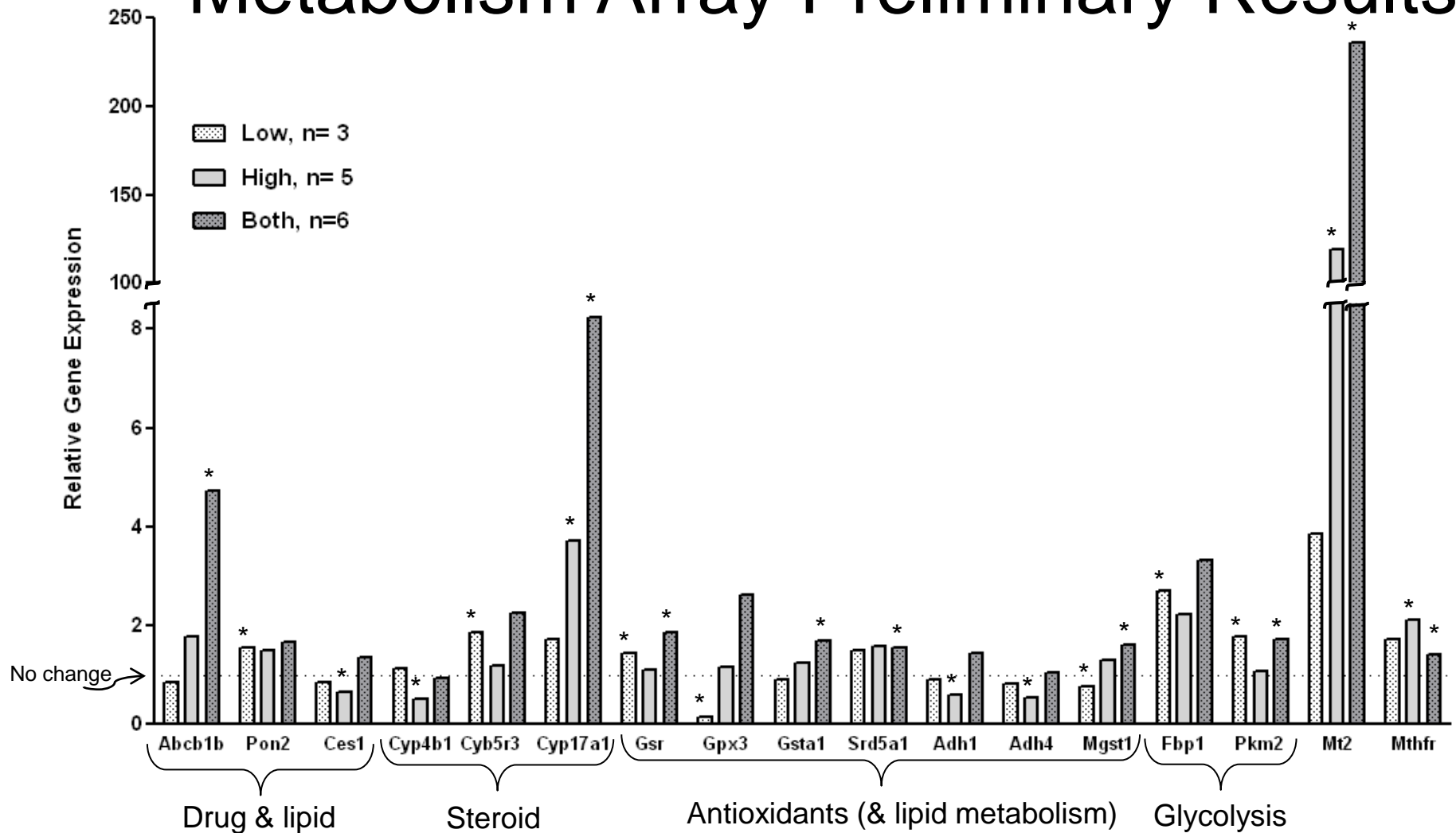
Number of genes changed:



	Low	High	Both	Unchanged
Drug Metabolism Genes (Changes up to 240 fold)	5	7	7	74
DNA Repair Genes (No changes over 3-fold)	6	14	4	77

Unchanged genes can be safely eliminated from future, more detailed investigations at the protein level.

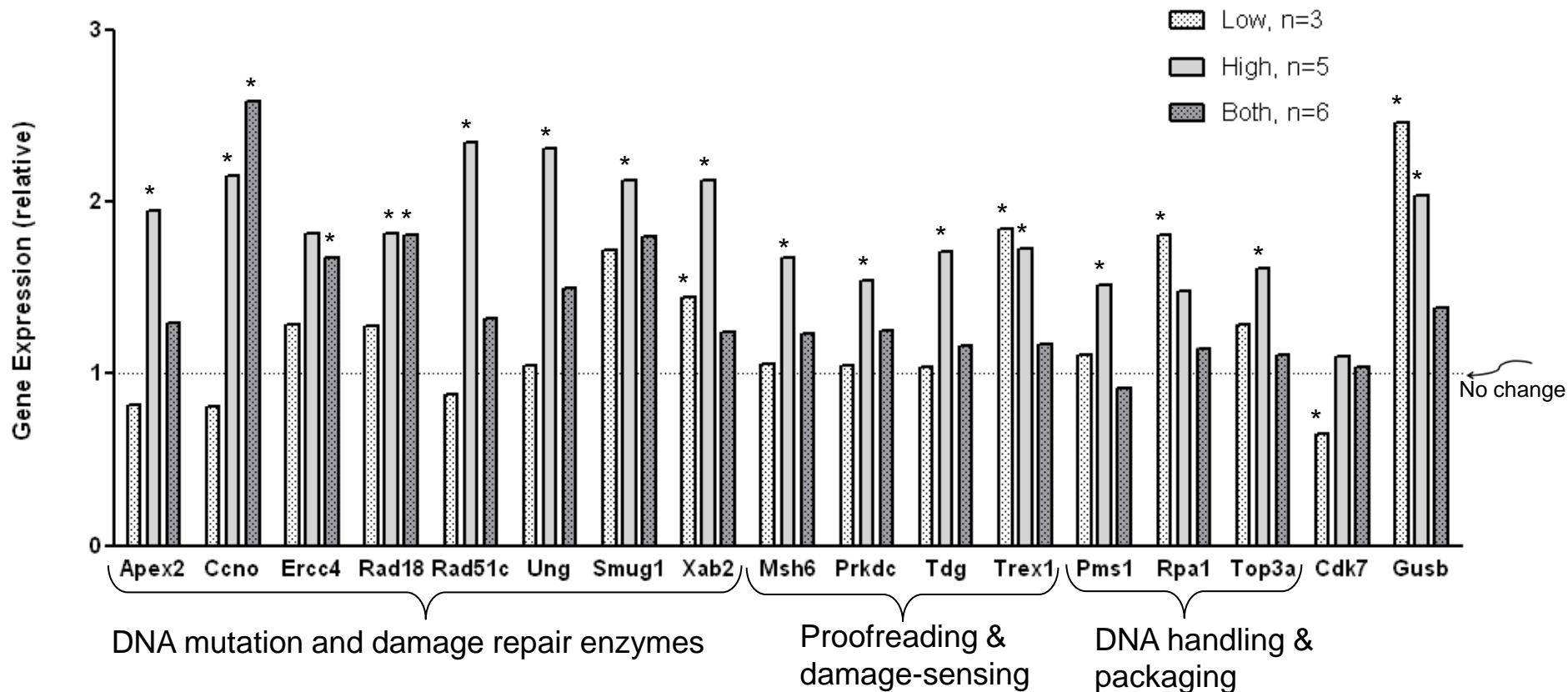
Metabolism Array Preliminary Results



Data are normalized gene expression relative to a set of reference genes (ActB, Hprt1, Gstm1, Gad1, Gapdh); deviations from the dotted line at $y=1$ indicate changes from gene expression measured in control animals. The data shown here are those whose gene expression changed by > 1.5 fold with $p < 0.05$ (compared to control; indicated by *) in at least one of the treatment groups (low, high or both). Data from 74 genes with no significant expression changes are not shown. Detailed analysis (including SEM and statistical analysis) is underway.

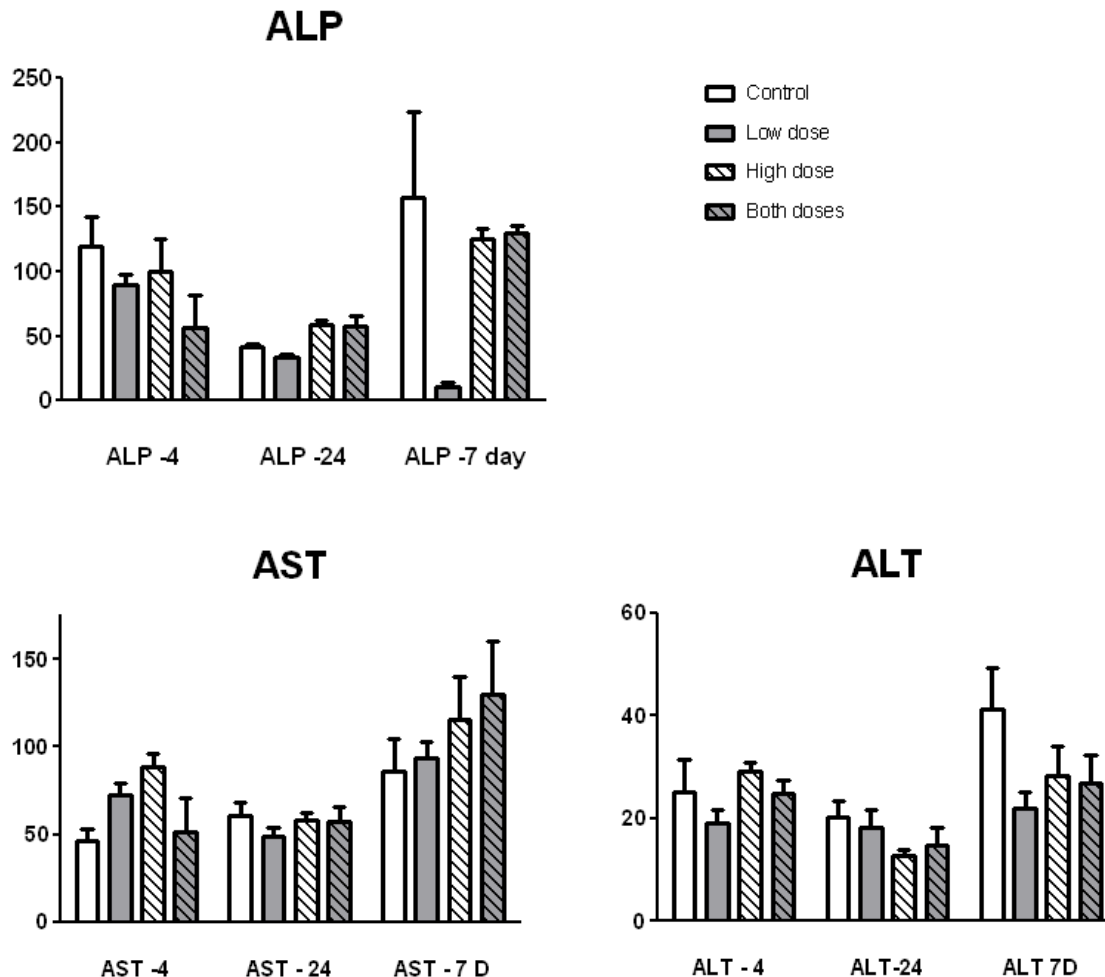
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DNA Repair Array Preliminary Results



Data are normalized gene expression relative to a set of reference genes (Rfc1, Ccnh, Atxn3, Actb); deviations from the dotted line at $y=1$ indicate changes from gene expression measured in control animals. The data shown here are those whose gene expression changed by > 1.5 fold with $p < 0.05$ (compared to control; indicated by *) in at least one of the treatment groups (low, high or both). Data from 77 genes with no significant expression changes are not shown. Detailed analysis (including SEM and statistical analysis) is underway.

Clinical liver function tests from serum

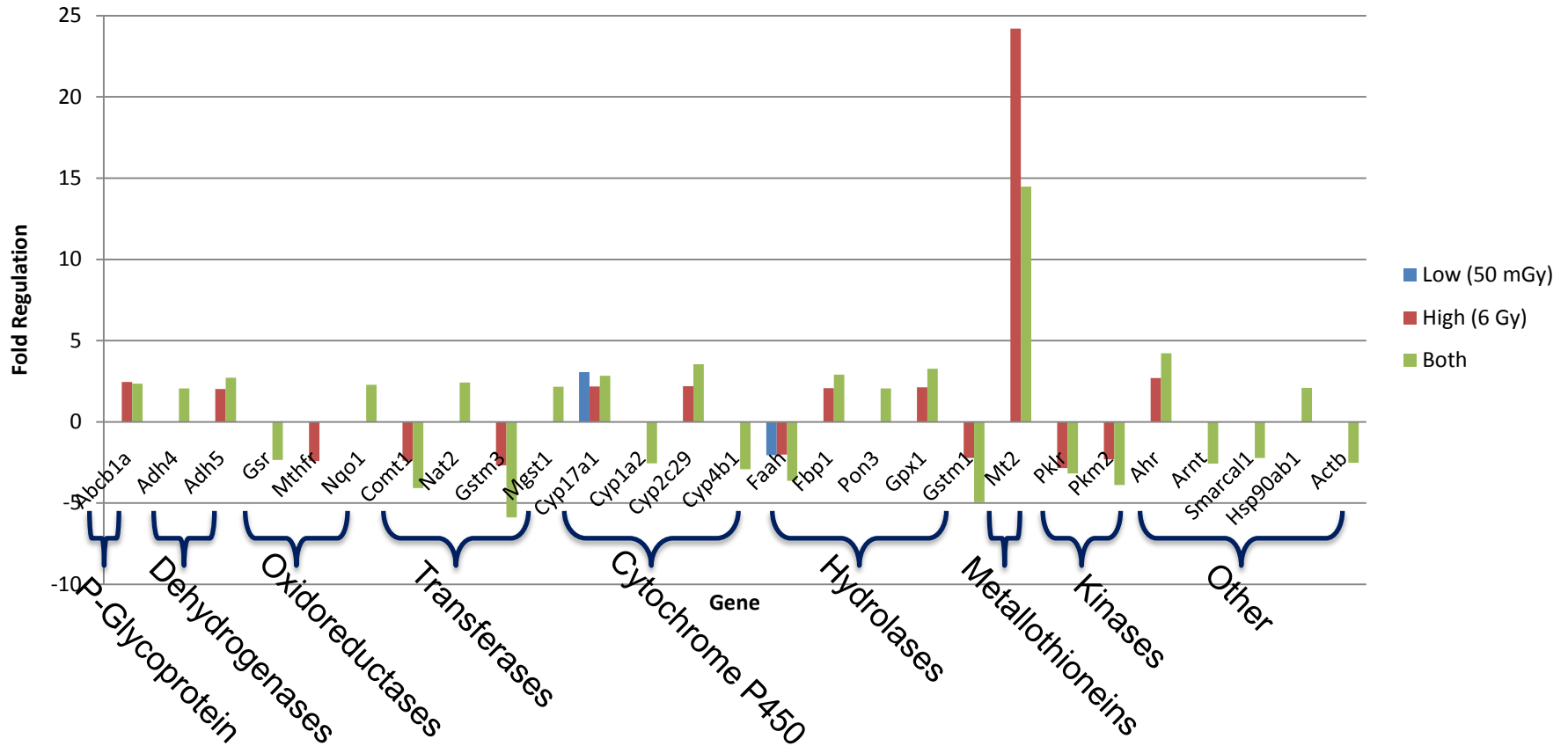


All measured values from liver function tests alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) are within normal ranges, although there ~~some are significant differences from controls (indicated by *)~~. The 4 hour experiment is the left group of columns on each graph; the 24 hour experiment is on the right. N=6 for all, except



Drug Metabolism Results

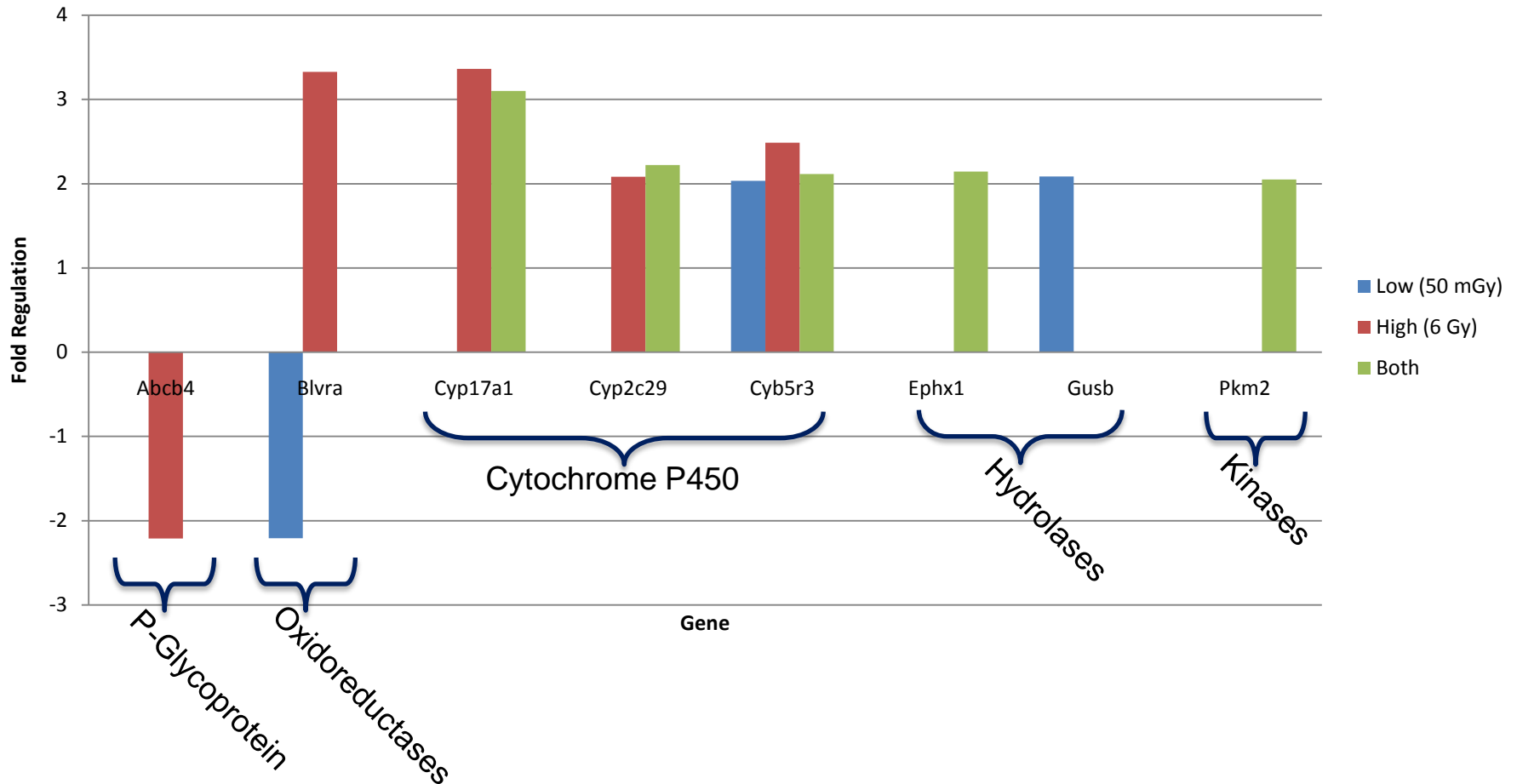
Gene Expression at 24 Hours





Drug Metabolism Results

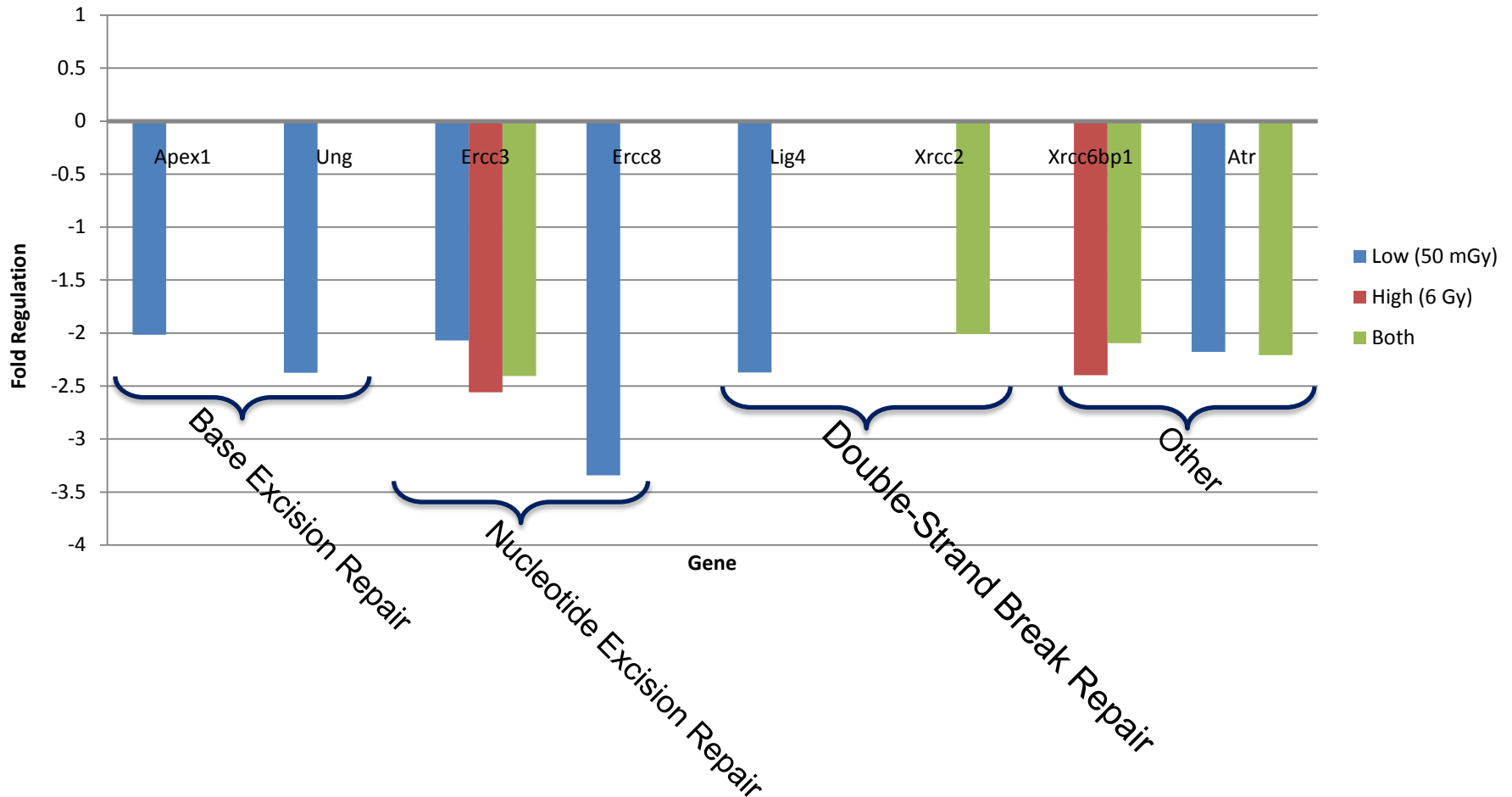
Gene Expression at 7 Days



DNA Repair Results



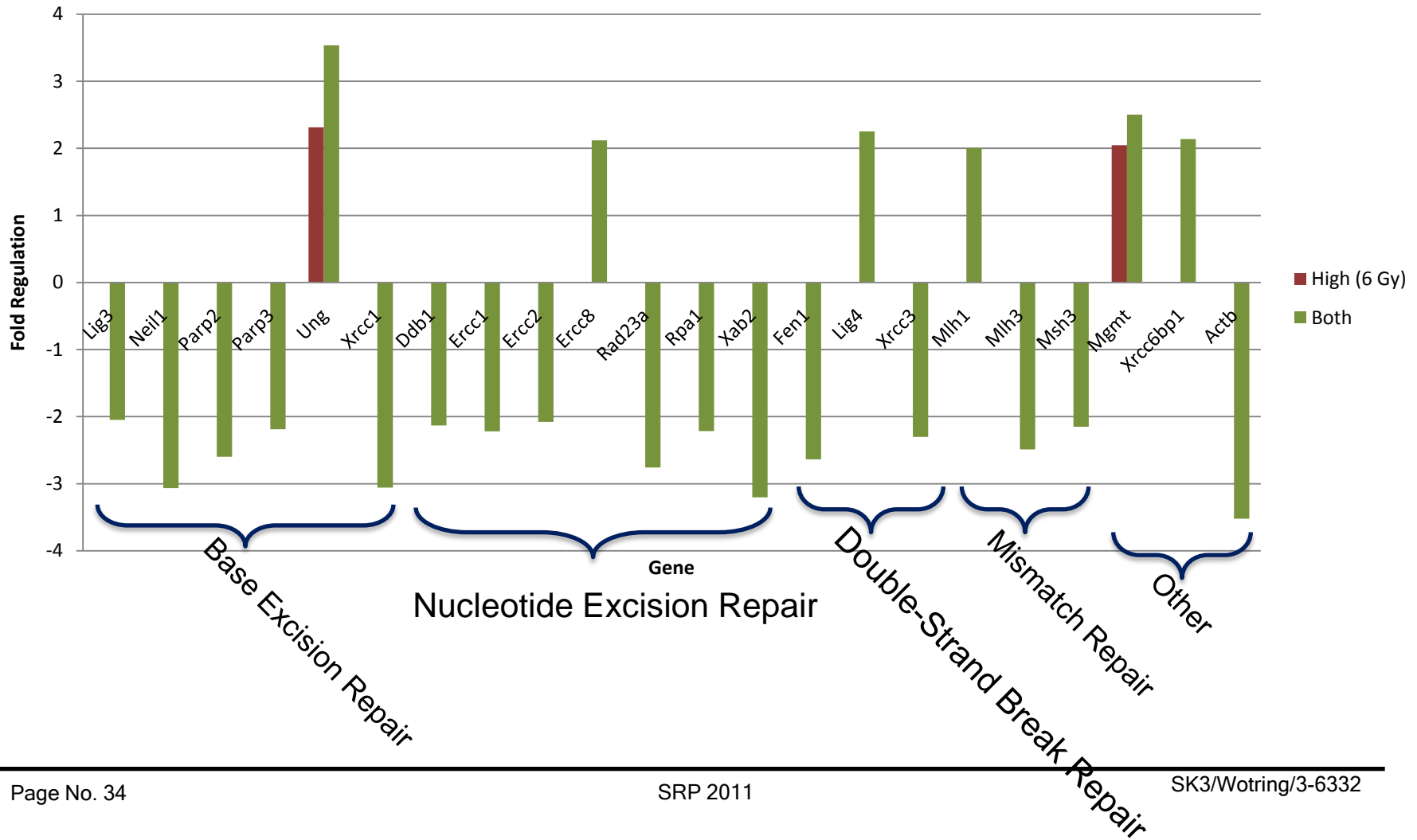
Gene Expression at 4 Hours





DNA Repair Results

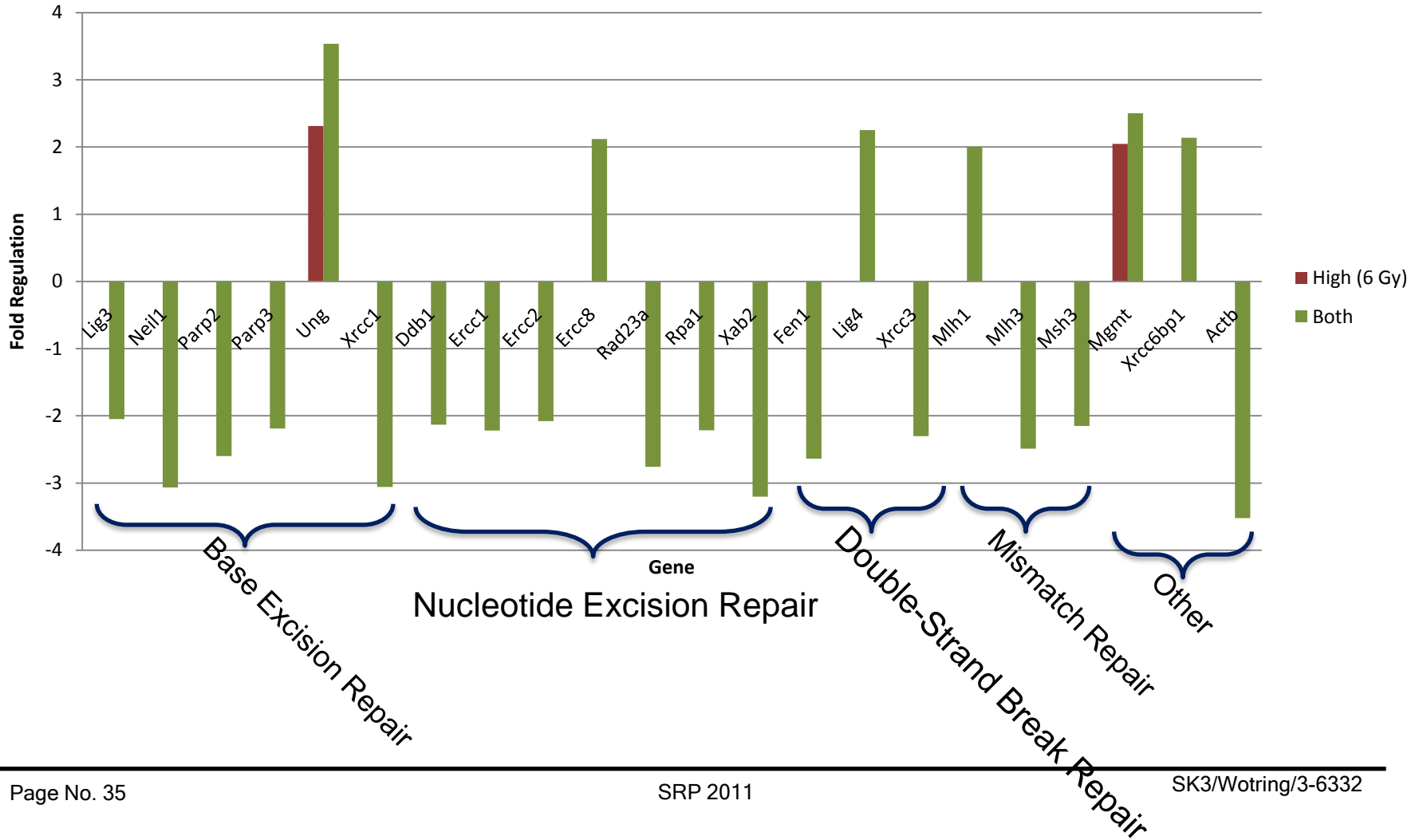
Gene Expression at 24 Hours



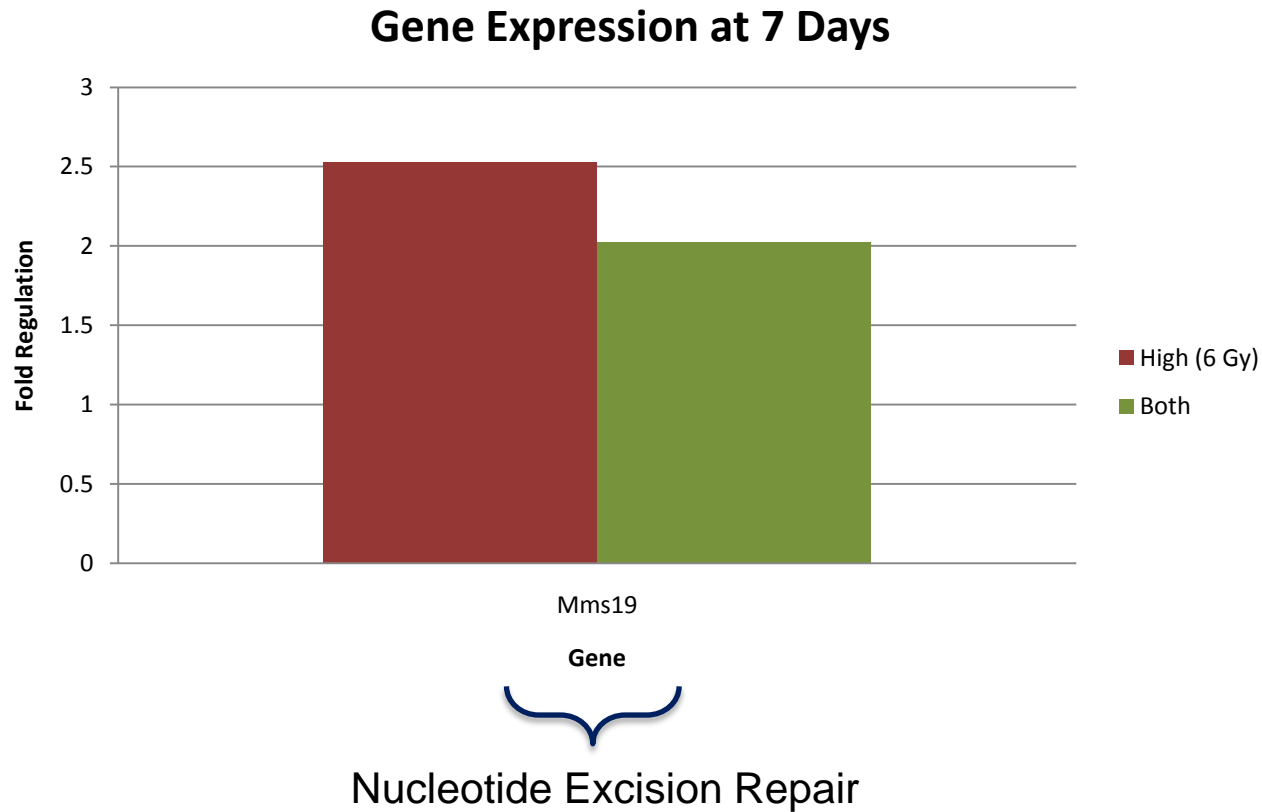


DNA Repair Results

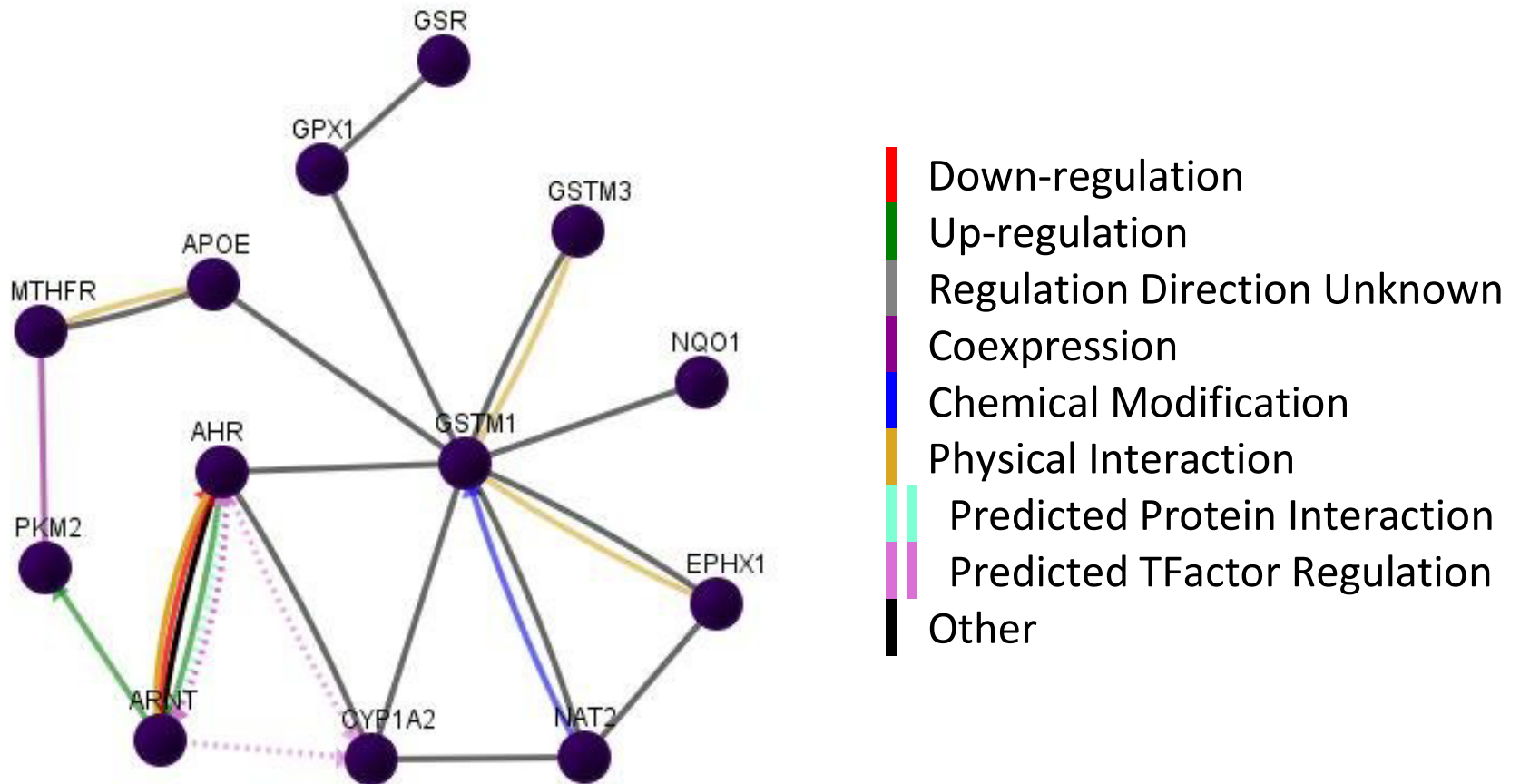
Gene Expression at 24 Hours



DNA Repair Results



Drug Metabolism Gene Relationships



DNA Repair Gene Relationships

